PI(4,5)P2 degradation by synaptojanin and/or a lipid-shaping function by endophilin might be sequentially and/or cooperatively required for the transition of shallow into deeply invaginated pits, the fission of vesicles from the donor membrane, the uncoating of vesicles, and for cytoskeletal interactions of endocytosing vesicles. Hence, it will be important, though difficult, to dissect when and where each individual enzymatic activity is required during endocytosis. As usual, obtaining some answers leads only to more questions.

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Glycine Transporters Not Only Take Out the Garbage, They Recycle

Two articles in the current issue of *Neuron* examine the consequences of deleting the two genes that encode glycine transporters. Interestingly, loss of glial transporters enhances while loss of presynaptic neuronal transporters reduces glycinergic transmission. These two opposing phenotypes resemble distinct human diseases characterized by dysfunction in glycinergic signaling.

After release, most neurotransmitters are removed from the vicinity of the synapse by specialized plasma membrane transporters. Like tiny garbage men, these integral membrane proteins perform a thankless task, clearing the refuse of neurotransmission from the synapse. In the absence of such cleanup, circuits become littered with excess neurotransmitter. Allowing neurotransmitters to pile up not only impedes signaling, it drains resources by permitting a valuable, reusable commodity to go to waste. In the delicate economy of the synapse, then, it is up to these garbage men to regulate the fine balance between recycling and consumption.

In order to meet these needs, multiple genes encode transporters for certain neurotransmitters, such as glutamate (five genes), GABA (four genes), and glycine (two genes). For the two glycine transporters GlyT1 and GlyT2, unique expression patterns suggest specialized functions. The neuronal transporter GlyT2 is largely confined to presynaptic terminals that secrete glycine, and so its localization matches the distribution of glycinergic neurons found mainly in the brain stem and spinal cord (Jursky and Nelson, 1995; Zafra et al., 1995). Because of this overlap, a prevailing view has been that GlyT2 is the principal pathway for reuptake of glycine released at glycinergic synapses. In comparison, a much broader expression pattern is observed for the glial isoform GlyT1 (Zafra et al., 1995). This transporter is located in regions of the brain not known to rely on glycinergic inhibition (cortex, hippocampus, thalamus), suggesting that it has another role. Since glycine is also known to act as a coagonist of excitatory NMDA receptors, it has been hypothesized that GlyT1 influences excitatory synaptic signaling by regulating ambient glycine concentrations (Smith et al., 1992; Berger et al., 1998; Chen et al., 2003). Thus, although the reasons for genetic redundancy are uncertain, it is appealing to think that individual transporters have specialized functions.

In this issue of *Neuron*, papers by Gomeza and Hülsmann et al. (Gomeza et al., 2003a) and Gomeza et al. (2003b) test these assumptions by generating two lines of knockout mice, one deficient in the glial isoform and the other lacking the neuronal isoform of glycine transporter. Avoiding the pitfalls of pharmacological manipulation, these studies provide the clearest view yet of specific roles played by glial and neuronal glycine uptake.

In the first of the two papers, Gomeza and Hülsmann et al. inactivate the glial transporter gene. Genotyping these homozygous mutant mice (*GlyT1*$^{-/-}$) confirmed that they were devoid of the wild-type allele. Further analysis showed that, while both GlyT1 transcript and protein were absent in the mutants, GlyT2 remained unaffected. As expected from the normal distribution of GlyT1, tissue originating from both forebrain and brain stem regions of *GlyT1*$^{-/-}$ mice exhibited significantly reduced uptake of radiolabeled glycine.

In the second paper, Gomeza et al. performed similar analyses on mice deficient in the neuronal glycine transporter. In these animals (*GlyT2*$^{-/-}$), glycine uptake was selectively impaired in brain stem and spinal cord, regions known to contain a large number of glycinergic synapses. Remarkably, neither the loss of GlyT1 nor of GlyT2 caused noteworthy changes in histology and synaptic protein expression. There seemed to be no increase in embryonic mortality, and homozygous mutant mice appeared grossly normal at birth.

Loss of either transporter severely shortened the lifespan of homozygous animals, but the increased mortality was due to very different mechanisms. *GlyT1*$^{-/-}$ mice showed impaired motor activity and died on the first day after birth. For the short time that they were alive, these animals responded lethargically to tactile stimuli and generally appeared hypotonic, observations that led the researchers to suspect wide-ranging neuromotor deficits. Dysfunction of motor activity extended to the respiratory system as plethysmographic recordings demonstrated that the breathing patterns of *GlyT1*$^{-/-}$ mice were severely depressed. The authors examined this issue in more detail by monitoring neuronal activity in acute slices of brain stem containing circuitry responsible for generating the respiratory rhythm. Extracellular recordings made from *GlyT1*$^{-/-}$ slices revealed a slowed and irregular rhythm, a condition rescued by application of the glycine receptor antagonist strychnine. Similar effects were seen in wild-type slices following application of glycine or pharmacological inhibition of GlyT1. Finally, whole-cell voltage-clamp recordings from hypoglossal neurons in *GlyT1*$^{-/-}$ slices revealed the appearance of a tonic glycine receptor current, an increase in the frequency of spontaneous inhibitory synaptic currents, and a slowing of their decay kinetics. Taken together, these findings convincingly argue that hyperactive glycinergic signaling causes the depressed respiratory function and, by extension, the shortened lifespan seen in *GlyT1*$^{-/-}$ mice. They also reveal an underappreciated role for glial glycine transporters at synapses previously believed to depend mainly on neuronal uptake.

Mice carrying the GlyT2 mutation lived a bit longer but eventually succumbed to a multitude of neuromotor ailments by the end of the second postnatal week. *GlyT2*$^{-/-}$ mutants displayed spastic muscle tone, tremor, and impaired righting response. Electrophysiological analysis of inhibitory activity in brain stem slices and culture preparations showed a reduction in the amplitudes of spontaneous and miniature glycinergic inhibitory currents, evidence that glycine release is depressed in *GlyT2*$^{-/-}$ animals. Because normal levels of glycine receptors were found at these synapses, the authors could rule out a reduction in postsynaptic sensitivity. Thus, data from *GlyT2*$^{-/-}$ mice imply that their phenotype results from diminished glycine concentration in the presynaptic terminal. These findings point to an essential role for GlyT2 in the recycling of glycine.

Through their careful comparison of GlyT-deficient mice, Gomeza and Hülsmann et al. and Gomeza et al. make a strong case that the two glycine transporter proteins are specialized to perform different physiological tasks. However, the unique cell-specific expression of transporters may not explain all of the physiological differences. Recent work has suggested that glial and neuronal transporters have slightly distinct functional properties. Based on measurements of steady-state energetics, Roux and Supplisson (2000) have shown that the glial transporter is less efficient at concentrating glycine than its neuronal counterpart. They propose that in brain regions where the two transporters are colocalized the glial transporter may serve as a glycine source rather than a sink by pumping glycine out of glia and into the extrasynaptic space (Supplisson and Roux, 2002). Evidence for reverse transport is not apparent in the work of Gomeza and Hülsmann et al. or Gomeza et al., nevertheless, future analyses of NMDA-mediated transmission in such brain regions may show that GlyT1 can also recycle, acting as a source for glycine under some conditions. Due to the poor viability of homozygous glycine transporter knockouts, these experiments may have to be performed on heterozygous animals.

Intriguingly, each line of mice presents symptoms that are very similar to rare human illnesses. For the hyperglycinergic *GlyT1*$^{-/-}$ mice, the analogous condition is glycine encephalopathy, while for the hypoglycinergic *GlyT2*$^{-/-}$ animals, it is hyperekplexia, or startle disease. Although neither of the human glycine transporter genes has been linked to either disease, mutations in glycine receptors (Rajendra and Schofield, 1995) and enzymes responsible for degrading glycine (Applegarth and Toone, 2001) have been implicated. It will be interesting to see whether dysfunctional glycine transporters also play a role in a subset of these conditions. Moreover, the opposing phenotypes described in these studies offer the possibility that each subtype of glycine transporter could serve as a specific clinical target. For instance, downregulating GlyT1 or GlyT2 could be used to either enhance or reduce glycinergic signaling, respectively. Of course, more information will be required to fully understand the physiology of glycine uptake. These mutant mice and their heterozygous progeny should prove invaluable in this effort. Hopefully, future experiments will enable scientists and physicians to better treat human diseases by directing glycine either to the recycling bin or garbage pail.

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Age-Related Memory Impairment: Is the Cure Worse than the Disease?

Aging impairs multiple memory systems. The neurochemical substrates of normal memory differ between memory systems. In this issue of *Neuron*, Ramos et al. find that activation of protein kinase A, which has been reported to improve hippocampal-dependent spatial memory in aged animals, has the opposite effect on prefrontal cortex-dependent working memory in aged animals.

One of the major discoveries of neuropsychology and behavioral neuroscience in the last 50 years is the existence of multiple memory systems. Different aspects of memory are affected by damage to different brain regions (Eichenbaum and Cohen, 2001). This research provides a foundation for understanding the neurobiological substrates of memory processing—in order to understand the cellular and molecular processes that underlie memory, one must know where to look.

Aging impairs both hippocampal-dependent and prefrontal-dependent memory function, as well as other cognitive domains (Gallagher and Rapp, 1997). Cognitive aging does not appear to be the result of a homogeneous disruption of brain function: age-related impairments in hippocampal-dependent memory appear, at least to some extent, to be independent of age-related impairments in prefrontal function (e.g., Barense et al., 2002). While acknowledging that there are theoretical uncertainties with the concepts of independent memory systems (Gaffan, 2002) and that these areas are involved in other aspects of memory and cognition besides episodic and working memory (e.g., Duncan and Owen, 2000), there is compelling physiological evidence for correlates of these two different processes within these two cortical areas. Hippocampal neurons encode memory "episodes," complex conjunctions of spatial and/or temporal information (e.g., Wood et al., 2000). Neurons in dorsolateral prefrontal cortex possess "memory fields" for spatial locations in monkeys performing a task requiring working memory for one of a number of spatial locations (e.g., Funahashi et al., 1989). Notably, short-term memory for spatial location does not require an intact hippocampus (Angeli et al., 1993), supporting the notion of some independence between these systems.

The localization of different memory processes in different cortical areas, and the understanding of their biochemical and physiological underpinnings, promises pharmacological therapies that can bolster neural mechanisms that fail in aging. Much progress in this regard has been made based on animal models of hippocampal-dependent memory function. For example, the identification of molecular pathways involved in long-term memory storage in the hippocampus has informed the development of agents that should improve hippocampal-dependent memory when it is impaired by aging. Compounds that inhibit cAMP breakdown, thereby increasing protein kinase A (PKA) activation, have been reported to improve physiological and behavioral indices of hippocampal-dependent memory in aged mice (Barad et al., 1998; Bach et al., 1999). If one assumes that age-related impairment in hippocampal-dependent memory processing in rodents is a good model for cognitive aging in humans, then this predicts that such agents will also benefit memory function in aged humans, possibly including those that have memory deficits stemming from pathological conditions associated with aging (such as Alzheimer's disease).

Although much has been learned about the neural bases of age-related memory impairment by studying neurobiological correlates of impaired hippocampal-dependent memory in aging, this approach neglects the contribution of other brain systems. Impaired prefrontal function also occurs in aging, and disruption of working memory and other aspects of executive function poses a challenge for aged individuals. Comparatively little is known about the biological substrates of age-related impairments in prefrontal memory function.

Because PKA activation improves hippocampal-dependent memory in aged rodents, it is important to understand the consequences of this treatment on other memory systems. In this issue of *Neuron*, Ramos et al. examined the effects of PKA activation and inhibition on prefrontal-dependent working memory in aged rats and monkeys. Earlier work from this group had shown that PKA activation in prefrontal cortex of young rats impaired working memory (Taylor et al., 1999), similar to the effect of overstimulation of dopamine D1 receptors (Zahrt et al., 1997). This is perhaps not surprising, as it is suggestive of a canonical U-shaped dose-response curve in which optimal dopamine receptor stimulation is necessary for normal working memory (Zahrt et al., 1997). However, the prediction for the situation in the aged brain is anything but clear. Given that dopamine levels in prefrontal cortex decrease in aging, it might be expected that stimulation of PKA activity would be beneficial to working memory. This would be a welcome state of affairs, because PKA stimulation would improve both prefrontal- and hippocampal-dependent memory.

The findings of Ramos et al. on PKA regulation and working memory in aged animals are in striking contrast to the effects of PKA activation on hippocampal-dependent spatial memory. Figure 1 illustrates a simplified schematic of PKA regulation, along with the points in the pathway targeted by the different pharmacological agents used by Ramos et al. Activation of PKA in prefrontal cortex of aged rats by direct infusion of Sp-CAMPS into the prefrontal cortex impairs working memory, whereas PKA inhibition by infusion of Rp-CAMPS facilitates it. These effects are more pronounced in rats that show greater impairment in performing the working memory task: thus, rats with more impaired prefrontal